

THAT WHICH IS CLAIMED:

1. A method for recovering a recombinant protein from plant tissue comprising steeping said plant tissue with a steeping solution so as to produce steep
5 water; wherein said plant tissue comprises at least one recombinant protein, and said recombinant protein is recovered in said steep water.
2. The method of claim 1, wherein said plant tissue is steeped for at least
10 about 1 hour.
3. The method of claim 1, wherein said plant tissue is steeped for at least
about 6 hours.
4. The method of claim 1, wherein said plant tissue is steeped for at least
15 about 12 hours.
5. The method of claim 1, wherein said plant tissue is steeped for at least
about 24 hours.
- 20 6. The method of claim 1, wherein said plant tissue is selected from the group consisting of seeds, fruits, tubers, roots, shoots, leaves, petioles, stems, and flowers.
7. The method of claim 1, wherein said steeping solution comprises
25 water.
8. The method of claim 7, wherein said steeping solution further
comprises at least one component selected from the group consisting of sulfur
dioxide, inorganic acids, organic acids, and salts.
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9. The method of claim 8, wherein said inorganic acids are selected from
the group consisting of sulfurous acid, sulfuric acid, phosphoric acid, nitrous acid,

nitric acid, hypochlorous acid, hydrochloric acid, carbonic acid, boric acid, and hydrofluoric acid.

10. The method of claim 8, wherein said organic acids are selected from
5 the group consisting of lactic acid, formic acid, succinic acid, malic acid, pyruvic acid, ascorbic acid, malonic acid, tartaric acid, oxalic acid, propionic acid, acetic acid, *n*-butyric acid, isobutyric acid, and citric acid.

11. The method of claim 8, wherein said salts are selected from the group
10 consisting of sodium acetate, calcium acetate, potassium acetate, ammonium acetate, magnesium acetate, sodium benzoate, sodium chloride, calcium chloride, potassium chloride, ammonium chloride, magnesium chloride, sodium sulfate, calcium sulfate, potassium sulfate, ammonium sulfate, magnesium sulfate, sodium nitrate, calcium
nitrate, potassium nitrate, ammonium nitrate, magnesium nitrate, sodium nitrite,
15 potassium nitrite, sodium carbonate, calcium carbonate, potassium carbonate, ammonium carbonate, magnesium carbonate, sodium phosphate, calcium phosphate, potassium phosphate, ammonium phosphate, and magnesium phosphate.

12. The method of claim 1 further comprising concentrating said steep
20 water.

13. The method of claim 1 further comprising isolating said recombinant
protein from said steep water by utilizing at least one technique selected from the
group consisting of including centrifugation, ultrafiltration, dialysis, gel-filtration
25 chromatography, ion-exchange chromatography, affinity chromatography, immunoaffinity chromatography, high-performance liquid chromatography, reversed-
phase high-performance liquid chromatography, ion-exchange high-performance
liquid chromatography, size-exclusion high-performance liquid chromatography,
high-performance chromatofocusing, hydrophobic interaction chromatography, one-
30 dimensional gel electrophoresis, two-dimensional gel electrophoresis, and capillary electrophoresis.

14. The method of claim 1 further comprising at least one secondary extraction of said plant tissue, or at least one part thereof.

15. The method of claim 14, wherein said plant tissue is a seed and said part is selected from the group consisting of an embryo, an endosperm, a germ, a degerminated seed, a seed coat, a tip cap, and a pericarp.

16. The method of claim 14, wherein said secondary extraction comprises use of said steep water.

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17. The method of claim 1, wherein said plant tissue is from a transgenic plant comprising a stably integrated nucleic acid construct optimized for recovery of said recombinant protein, wherein said nucleic acid construct comprises a nucleotide sequence encoding said recombinant protein operably linked to a promoter that drives expression in a plant cell.

18. The method of claim 17, wherein said nucleotide sequence encodes a protein selected from the group consisting of brazzein, avidin, streptavidin, aprotinin, β -glucuronidase, alkaline phosphatase, insulin, bovine somatotropin, human growth hormone, fibrinogen, thrombin, factor IX, factor XIII, serum albumin, plasma proteins, protein C, invertase, superoxide dismutase, catalase, urease, lysozyme, lactase, glucose isomerase, α -amylase, glucoamylase, pullulanase, isoamylase, β -glucanase, xylanase, papain, trypsin, chymotrypsin, pepsin, proteases, protease inhibitors, esterases, peroxidases, hydrolases, phosphatases, kinases, ribonucleases, deoxyribonucleases, antibodies, phytases, lipases, phospholipases, cellulases, hemicellulases, pectinase, peptide hormones, insecticidal proteins, enzymes, and fusion proteins.

19. The method of claim 17, wherein said promoter is selected from the group consisting of tissue-preferred, seed-preferred, endosperm-preferred, embryo-preferred, inducible, chemical-regulated, and constitutive promoters.

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20. The method of claim 1, wherein said recombinant protein is selected from the group consisting of brazzein, avidin, streptavidin, aprotinin, β -glucuronidase, alkaline phosphatase, insulin, bovine somatotropin, human growth hormone, fibrinogen, thrombin, factor IX, factor XIII, serum albumin, plasma proteins, protein C, invertase, superoxide dismutase, catalase, urease, lysozyme, lactase, glucose isomerase, α -amylase, glucoamylase, pullulanase, isoamylase, β -glucanase, xylanase, papain, trypsin, chymotrypsin, pepsin, proteases, protease inhibitors, esterases, peroxidases, hydrolases, phosphatases, kinases, ribonucleases, deoxyribonucleases, antibodies, phytases, lipases, phospholipases, cellulases, hemicellulases, pectinase, peptide hormones, insecticidal proteins, enzymes, and fusion proteins.
21. The method of claim 1, wherein said plant tissue is from a monocot.
22. The method of claim 21, wherein said monocot is selected from the group consisting of corn, wheat, oats, millet, sorghum, rice, barley, rye, palms, coconut, banana, duckweed, onion, garlic, and sugarcane.
23. The method of claim 1 where said plant tissue is from a dicot.
24. The method of claim 23, wherein said dicot is selected from the group consisting of soybean, canola, oilseed rape, cotton, sunflower, safflower, peas, flax, tobacco, beans, fava beans, mung beans, chickpeas, cowpeas, lentils, lupines, alfalfa, potato, tomato, peppers, sugar beet, cassava, cocoa, carrot, cabbage, broccoli, cauliflower, lettuce, sweet potato, melons, watermelon, squashes, cucumber, peanut, apple, citrus, almond, olive, avocado, mango, papaya, cashew, coffee, guava, and grapes.
25. A method for recovering a recombinant protein from a seed comprising steeping said seed, or at least one part thereof, with a steeping solution so as to produce steep water; wherein said seed comprises at least one recombinant protein and said recombinant protein is recovered in said steep water.

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26. The method of claim 25, wherein said seed or said part is steeped for at least about 1 hour.

27. The method of claim 25, wherein said seed or said part is steeped for at least about 6 hours.

28. The method of claim 25, wherein said seed or said part is steeped for at least about 12 hours.

29. The method of claim 25, wherein said seed or said part is steeped for at least about 24 hours.

30. The method of claim 25, wherein said seed is a corn kernel.

31. The method of claim 30, wherein, following said steeping, said seed or said part is suitable for wet-milling.

32. The method of claim 25, wherein said steeping solution comprises water.

33. The method of claim 32, wherein said steeping solution further comprises at least one component selected from the group consisting of sulfur dioxide, inorganic acids, organic acids, and salts.

34. The method of claim 25, wherein said seed is from a transgenic plant comprising a stably integrated nucleic acid construct optimized for recovery of said recombinant protein, wherein said nucleic acid construct comprises a nucleotide sequence encoding said recombinant protein operably linked to a promoter that drives expression in a plant cell.

35. The method of claim 25, wherein said recombinant protein is selected from the group consisting of brazzein, avidin, streptavidin, aprotinin, β -glucuronidase, alkaline phosphatase, insulin, bovine somatotropin, human growth hormone,

fibrinogen, thrombin, factor IX, factor XIII, serum albumin, plasma proteins, protein C, invertase, superoxide dismutase, catalase, urease, lysozyme, lactase, glucose isomerase, α -amylase, glucoamylase, pullulanase, isoamylase, β -glucanase, xylanase, papain, trypsin, chymotrypsin, pepsin, proteases, protease inhibitors, esterases, 5 peroxidases, hydrolases, phosphatases, kinases, ribonucleases, deoxyribonucleases, antibodies, phytases, lipases, phospholipases, cellulases, hemicellulases, pectinase, peptide hormones, insecticidal proteins, enzymes, and fusion proteins.

- 10 36. A method for producing a recombinant protein comprising:
- (a) providing a plant, or at least one cell thereof, with at least one nucleic acid construct comprising a nucleotide sequence encoding said recombinant protein operably linked to a promoter that drives expression in a plant cell;
- 15 (b) obtaining plant tissue from said plant, or from a descendant of said plant, wherein said plant tissue comprises said recombinant protein; and
- (c) steeping said plant tissue with a steeping solution so as to produce steep water, wherein said recombinant protein is recovered in said steep water.

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37. The method of claim 36, wherein said nucleic acid construct is optimized for recovery of said recombinant protein.

25 38. The method of claim 37, wherein said promoter is selected from the group consisting of tissue-preferred, seed-preferred, endosperm-preferred, embryo-preferred, inducible, chemical-regulated, and constitutive promoters.

30 39. The method of claim 37, wherein said nucleic acid construct further comprises at least one operably linked element selected from the group consisting of an enhancer, a transcriptional terminator region, a translational terminator region, an intron, exon-intron splice site signals, transposon-like repeats, a translational leader, a polyadenylation signal, and a nucleotide sequence encoding a signal peptide

40. The method of claim 37, wherein said coding sequence is manipulated to alter the amino acid sequence of said recombinant protein.
41. The method of claim 37, wherein said coding sequence is codon optimized.
42. The method of claim 36, wherein said steeping solution comprises water.
43. The method of claim 36, wherein said recombinant protein is selected from the group consisting of brazzein, avidin, streptavidin, aprotinin, β -glucuronidase, alkaline phosphatase, insulin, bovine somatotropin, human growth hormone, fibrinogen, thrombin, factor IX, factor XIII, serum albumin, plasma proteins, protein C, invertase, superoxide dismutase, catalase, urease, lysozyme, lactase, glucose isomerase, α -amylase, glucoamylase, pullulanase, isoamylase, β -glucanase, xylanase, papain, trypsin, chymotrypsin, pepsin, proteases, protease inhibitors, esterases, peroxidases, hydrolases, phosphatases, kinases, ribonucleases, deoxyribonucleases, antibodies, phytases, lipases, phospholipases, cellulases, hemicellulases, pectinase, peptide hormones, insecticidal proteins, enzymes, and fusion proteins.
44. The method of claim 36, wherein said plant tissue is from a monocot.
45. The method of claim 44, wherein said monocot is selected from the group consisting of corn, wheat, oats, millet, sorghum, rice, barley, rye, palms, coconut, banana, duckweed, onion, garlic, and sugarcane.
46. The method of claim 36 where said plant tissue is from a dicot.
47. The method of claim 47, wherein said dicot is selected from the group consisting of soybean, canola, oilseed rape, cotton, sunflower, safflower, peas, flax, tobacco, beans, fava beans, mung beans, chickpeas, cowpeas, lentils, lupines, alfalfa, potato, tomato, peppers, sugar beet, cassava, cocoa, carrot, cabbage, broccoli, cauliflower, lettuce, sweet potato, melons, watermelon, squashes, cucumber, peanut, apple, citrus, almond, olive, avocado, mango, papaya, cashew, coffee, guava, and grapes.